

Effects of Environmental Agents on the Attainment of Puberty: Considerations When Assessing Exposure to Environmental Chemicals in the National Children's Study

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The apparent decline in the age at puberty in the United States raises a general level of concern because of the potential clinical and social consequences of such an event. Nutritional status, genetic predisposition (race/ethnicity), and environmental chemicals are associated with altered age at puberty. The Exposure to Chemical Agents Working Group of the National Children's Study (NCS) presents an approach to assess exposure for chemicals that may affect the age of maturity in children. The process involves conducting the assessment by life stages (i.e., *in utero*, postnatal, peripubertal), adopting a general categorization of the environmental chemicals by biologic persistence, and collecting and storing biologic specimens that are most likely to yield meaningful information. The analysis of environmental samples and use of questionnaire data are essential in the assessment of chemicals that cannot be measured in biologic specimens, and they can assist in the evaluation of exposure to nonpersistent chemicals. Food and dietary data may be used to determine the extent to which nutrients and chemicals from this pathway contribute to the variance in the timing of puberty. Additional research is necessary in several of these areas and is ongoing. The NCS is uniquely poised to evaluate the effects of environmental chemicals on the age at puberty, and the above approach will allow the NCS to accomplish this task. **Key words:** children, environmental chemicals, exposure assessment, hormonally active agents, National Children's Study, puberty. *Environ Health Perspect* 113:1100–1107 (2005). doi:10.1289/ehp.7615 available via <http://dx.doi.org/> [Online 12 May 2005]

The purpose of the National Children's Study (NCS) is to evaluate the health risks to children in this country from environmental exposures by using a longitudinal cohort design (Children's Health Act of 2000). This effort is expected to improve our children's health by mitigating these health risks in our society. The approach of the NCS is to centralize its activities around hypotheses that evaluate the relationship between a wide array of environmental exposures (i.e., chemical, biologic, physical, and psychosocial factors) and priority health outcomes. The list of priority outcomes includes pregnancy, neurodevelopmental injury, asthma, and obesity and physical development. One of several NCS hypotheses addressing these concerns seeks to evaluate the effects of environmental agents on the age at puberty, which was discussed at an inter-work group meeting co-chaired by the Nutrition, Growth, and Pubertal Development Working Group and the Exposure to Chemical Agents Working Group (Baltimore, Maryland, 17–18 December 2002). This article presents a brief background on the factors associated with the age of maturity and discusses the assessment for exposure to environmental chemical agents in the developing child by using a life-stage approach. The articles in this mini-monograph describe the roles and efforts of the Exposure to Chemical Agents Working Group in the NCS. Portions of this discussion may refer the reader to these other articles for additional information.

Recent evidence suggests that puberty in U.S. children is starting at an earlier age compared with previous years (Anderson et al. 2003; Freedman et al. 2003; Herman-Giddens et al. 1997, 2001). This is of general interest because the extent to which this is occurring in this population has not been well characterized and because such findings have potential influences within our society. In cross-sectional studies of populations in the United States from 1988 through 1994, it is reported that the mean age of onset for breast development for girls was 9.5–9.7 years, which is approximately 1–2 years earlier than the observations of previous investigators (Lee et al. 2001). This approximates to 14% of the population achieving Tanner stage 2 or greater for breast development at the age of 8 years (Lee et al. 2001). In U.S. boys sampled from 1988 through 1994, the median age of onset for genital development ranged from 9.5 to 10.4 years, depending on race/ethnicity, which is about 1 year earlier than that previously described (Herman-Giddens et al. 2001). This corresponded to about 32–58% of the boys attaining Tanner stage 2 at 9 years of age. These observed changes in pubertal development are not unique to the United States but also have been noted in other countries (Huen et al. 1997; Karlberg 2002; Parent et al. 2003; Proos et al. 1993; Viner 2002). To define properly the significance of these findings to the population, more refined investigations are needed to characterize the time trend in pubertal development because

of the limitations in study design of these prior reports (Lee et al. 2001; Reiter and Lee 2001; Viner 2002). Nutrition, genetic predisposition, and environmental chemical exposure are factors associated with pubertal change that can be evaluated during this process.

The factors associated with altered pubertal development are numerous (Parent et al. 2003). When early onset of puberty was originally noted in immigrant children, it was attributed to improved nutrition and well-being. Although these remain the primary factors determining the onset and progression of puberty, there are other considerations. These include genetic predisposition or host susceptibility, and environmental chemical exposure. Certain populations may be susceptible to variations in pubertal development based on observations among racial/ethnic groups (Anderson et al. 2003; Freedman et al. 2002; Sun et al. 2002; Wu et al. 2002). African-American girls were reported to develop either thelarche or pubarche at about the mean age of 8.8 years, 1–2 years earlier than non-Hispanic white girls (Herman-Giddens et al. 1997). This trend in race/ethnic groups for the age of onset for puberty was noted in boys as well (Herman-Giddens et al. 2001; Sun et al. 2002). Although obesity was associated with the early onset of puberty in these racial/ethnic groups, it was less of a determining variable in African-American girls, suggesting the involvement of other factors (Kaplowitz et al. 2001). In a study of serum leptin levels in girls 8–17 years of age, African-Americans girls were noted to have higher leptin levels than Caucasians after controlling for obesity, age, and serum insulin levels (Wong et al. 1998). Although serum leptin was shown to increase before gonadotropin levels in girls and boys (Garcia-Mayor et al. 1997),

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the signaling pathway for leptin in the development of puberty is not known, and further work is necessary to define this mechanism and the difference in leptin levels among racial/ethnicity groups (Danadian et al. 1999; Ruhl et al. 2004).

The early onset of puberty is clinically and socially important to the population. The early onset of thelarche is associated with an early diagnosis of breast cancer in susceptible populations (Hamilton and Mack 2003) and adult obesity (Biro et al. 2003). Breast cancer is associated with an early age of onset of menarche as well. Girls attaining menarche after 13 years of age were observed to have a one-third decreased risk [multivariate relative risk = 0.66; 95% confidence interval (CI), 0.44–0.99] for breast cancer compared with those attaining menarche at a younger age (Garland et al. 1998). This effect also was characterized by defining a 2-year delay in the age of menarche to a 10% decrease in risk for breast cancer (Hsieh et al. 1990). These observations imply the need for additional surveillance for these measures because they can affect diagnostic evaluation (Chalumeau et al. 2002; Dorn et al. 1999; Kaplowitz and Oberfield 1999). The social consequence is that children will need to learn how to adapt to their maturing bodies at an earlier age, and our public school system will need to re-evaluate the timing and structure of health education classes because the early onset of puberty has been associated with risky and unhealthy behaviors (Orr and Ingersoll 1995; Simon et al. 2003).

Environmental Chemicals

Several environmental chemicals are known to have hormonal activity, and their effects are well demonstrated in animal studies (Goldman et al. 2000; Stoker et al. 2000). The hormonal effects of these chemicals can be categorized by estrogenic, antiestrogenic, androgenic, antiandrogenic, and thyroidal activities. Depending on a chemical's hormonal activity, onset of puberty may be either delayed or accelerated. There is also experimental evidence demonstrating that chemical exposures during gestation can result in altered mammary gland development (Nikaido et al. 2004; Rayner et al. 2004) and age of pubertal onset (Casanova et al. 1999; Nikaido et al. 2004; Tou et al. 1998). The effects of these chemicals on the growth, development, and reproductive health of people are largely unknown because this remains a relatively new area of investigation. The chemical class that is best described in this area is polychlorinated biphenyls (PCBs). PCBs and their hydroxylated metabolites are known for their effects on the thyroid regulatory pathway, which can affect neurodevelopment (Jacobson and Jacobson 1996; Meerts et al. 2002). In addition to PCBs, several other chemicals with

hormonal activity are associated with health effects after exposure, some of which are discussed below (Table 1). (Note that the discussion of these chemicals does not imply that the NCS has determined that these chemicals either cause these health effects or will be included in the NCS.)

Environmental lead was recently observed to be associated with delayed age of onset of puberty in girls in the United States (Selevan et al. 2003; Wu et al. 2003). In a cross-sectional survey of 1,706 girls 8 through 16 years of age during 1988 through 1994, increased blood lead level was associated with a decreased likelihood for the attainment of either pubarche or menarche but not thelarche (Wu et al. 2003). Girls with lead levels in the ranges of 2.1–4.9 and 5–21.7 µg/dL were approximately 50 and 80%, respectively, less likely to reach these measures of puberty than those with lead levels between 0.7 and 2.0 µg/dL. In younger girls (8 through 12 years of age), the percentage of girls attaining pubarche by age decreased with increasing blood lead levels. When the same population was analyzed by racial/ethnicity groups, a higher blood lead level (3 vs. 1 µg/dL) in African-American girls was associated with delayed onset of pubarche, thelarche, and menarche by 2–4 months (Selevan et al. 2003). There also was a decreased likelihood of approximately 60% for the attainment of a successive Tanner stage for breast and pubic hair development in African-American girls. Similar delays in thelarche and pubarche were observed in Mexican-American girls, except the delays were smaller in magnitude. No differences in blood lead levels were observed in pubertal outcomes in non-Hispanic white girls. Underlying differences in growth or hormonal regulation among the racial/ethnic groups may explain the observed pubertal effects in association with lead exposure (Wong et al. 1998).

Chronic exposure to lead was demonstrated to affect the hypothalamo-pituitary-gonadal axis by altering serum levels of gonadotropic and androgenic hormones. In occupationally exposed males, prolonged lead exposure at the workplace was associated with decreased serum testosterone (Rodamilans et al. 1988). Serum luteinizing hormone levels were found to be lower both in occupational males (McGregor and Mason 1990) and in children 11 through 13 years of age from the general population (Vivoli et al. 1993) with high lead levels. The effects of exposure to environmental lead on the levels of estrogens, androgens, and gonadotropins in younger children and their pubertal development remain to be determined. Future studies in this area need to pay particular attention to the timing of exposure during development and the contribution of race/ethnicity to such findings.

The effects of the exposure to persistent organohalogen chemicals on maturation have been evaluated in several epidemiologic investigations. Two studies evaluated the contribution of *in utero* and postnatal exposure to these chemicals. Polybrominated biphenyls (PBBs) were found to be associated with premature thelarche in girls who were breast-fed by mothers with high serum PBB levels (≥ 7 µg/L) resulting from exposure to milk from cows given contaminated feed (Blanc et al. 2000). However, in girls who were not breast-fed by their PBB-exposed mothers, the maternal serum PBB level was not associated with early breast development, thus suggesting the contribution of postnatal exposure to altered pubertal development in this setting. There also was an increased risk (adjusted hazard ratio, 3.70; 95% CI, 1.41–9.71) for the earlier onset of menarche in girls breast-fed by mothers with high serum PBB levels compared with girls who were not breast-fed by mothers with low serum PBB levels (less than or equal to limit of detection). In the North Carolina Infant Feeding Study (1978 through 1982), *in utero* exposure to either PCBs or 1,1'-(2,2-dichloroethenylidene)-bis[4-chlorobenzene] [*p,p'*-DDE, a metabolite of dichlorodiphenyltrichloroethane (*p,p'*-DDT)] was not associated with the time to attainment of the Tanner stages in boys and girls (Gladden et al. 2000). However, increasing human milk levels of PCBs and *p,p'*-DDE were associated with increased body weight in girls and boys, respectively. Other studies evaluating the effects of exposure to PCBs and dioxin-like compounds (Den Hond et al. 2002) and to endosulfan (Saiyed et al. 2003) on the timing of pubertal milestones found either no effects or delayed effects, which varied by gender. These latter reports were limited by either the contribution of potential risk factors (e.g., congenital disorders) to the findings or their inability to relate their findings to prenatal versus postnatal exposure because the participants were evaluated only during puberty.

Finally, nonpersistent chemicals such as phthalates (Colon et al. 2000) and phytoestrogens have been investigated for their association with altered time of onset of puberty as well. Phytoestrogens are naturally occurring environmental chemicals found in fruits, vegetables, and legumes (e.g., soybeans) and have notable estrogenic activity. In a case-control study, the intake of soy-based formula was associated with an increased likelihood of premature thelarche in children younger than 2 years but not in children diagnosed with thelarche beyond this age (Freni-Titulaer et al. 1986). This suggests that various factors were most likely contributing to early breast development in this study population, depending upon the age of onset of the disorder. In both these areas of investigation, additional work is needed to clarify

these earlier findings because of either their small sample sizes or the current availability of improved methodology (e.g., biomonitoring) in exposure assessment.

Despite the findings of the various studies presented in the preceding section, the extent to which environmental chemicals contribute to changes in human pubertal development remains largely unknown. This is because of limitations in the designs of the investigations from which these observations were made, the various approaches used in conducting exposure assessment, and the few number of chemicals studied to date that are related to populations. A focused investigation could

attempt to answer some of the questions regarding environmental chemical exposure and maturation. For example, what is the significance to pubertal development of the timing of exposure to a chemical during a child's growth? Current studies in this area do not allow for such conclusions because of cross-sectional or retrospective design.

The observations from either cross-sectional or retrospective study designs are difficult to interpret as to the timing of chemical exposure during a child's development, and use less than perfect models to assess for overall exposure when a single level of a chemical in a biologic matrix is available. An example of

when the latter situation becomes challenging is with human milk. When infant exposure is assessed from human milk consumption, both the changing level of the chemical in the milk during the breast-feeding period and the duration of breast-feeding must be considered. The timing of exposure to a chemical is extremely difficult to ascertain from these study designs. This is because the random sampling of blood or urine from an individual does not allow for a determination of when the exposure occurred, which could have been at any time before the procurement of the specimen. However, exposures to apparent events (e.g., the industrial chemical explosion in

Table 1. Human studies on the effects of environmental chemical exposure on pubertal development.

| Chemical | Reference | Developmental window or route of exposure assessed | Exposure measure | Level of exposure | Associated health effects | |
|-----------------------|-------------------------------------|--|--|---|--|--|
| | | | | | Males | Females |
| <i>p,p'</i> -DDE | Gladen et al. 2000 | Intrauterine | Milk level at birth | Median, 2.4 µg/g lipids Range, 0.3–23.8 µg/g lipids | No effect on attainment of puberty by DDE level from intrauterine exposure Increased height and body weight at puberty with increasing DDE level from intrauterine exposure | No effect on time of onset, height, or body weight at puberty by DDE level from intrauterine exposure |
| | | Postnatal (breastfeeding) | Lactational exposure index | | No effect on attainment of puberty by DDE level from lactational exposure | |
| | Krstevska-Konstantinova et al. 2001 | Puberty | Serum level at or postpuberty | Immigrant/adopted, 1.04 µg/L (median) Immigrant/nonadopted, 1.20 µg/L (median) Native, 0.13 µg/L (median) | Immigrant children with idiopathic precocious puberty had higher DDE levels than native children with precocious puberty; adoption status was not associated with DDE level | |
| Dioxin-like compounds | Den Hond et al. 2002 | Puberty | Serum level at puberty | Exposures: range of means, 0.11–0.21 ng TEQ/L | No effect on likelihood to attain either genital maturity or pubarche by TEQ level | Decreased likelihood to attain thelarche with increasing TEQ level |
| Endosulfan | Saiyed et al. 2003 | Puberty | Serum level at puberty | Exposed, 7.47 ± 1.19 µg/L (mean ± SD) Nonexposed, 1.37 ± 0.40 µg/L (mean ± SD) | Delayed pubarche and genital maturity with exposure | |
| Lead | Selevan et al. 2003 | Puberty | Blood level at puberty | Non-Hispanic whites: geometric mean, 1.4 µg/dL; 95% CI, 1.2–1.5 | | No effect on pubarche, menarche, or thelarche by lead level for non-Hispanic whites |
| | | | | African Americans: geometric mean, 2.1 µg/dL; 95% CI, 1.9–2.3 | | Delayed pubarche, thelarche, and menarche with increasing lead level for African Americans |
| | | | | Mexican Americans: geometric mean, 1.7 µg/dL; 95% CI, 1.6–1.9 | | Delayed pubarche and thelarche with increasing lead level for Mexican Americans |
| | | | | | | Decreased height with increasing lead level, regardless of race/ethnicity |
| | Wu et al. 2003 | Puberty | Blood level at puberty | Range, 0.7–21.7 µg/dL | | Delayed pubarche and menarche with increasing lead level, no effect on attainment of thelarche by lead level |
| PBBs | Blanck et al. 2000 | Intrauterine | Extrapolated maternal serum level postevent ^b | Mean ± SD, 17.3 µg/L ± 107.8; range, ND ^a to 1,142 µg/L | | Accelerated attainment of menarche with increasing intrauterine PBB level and breast-feeding |

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Seveso, Italy, in 1976) in combination with elevated levels are exceptions. Timing is more of a problem for a persistent chemical with a long half-life than for a nonpersistent chemical. This problem can make it difficult to assess either the occurrence of subacute exposures or the source of the exposure when location has changed. The lack of consideration for these concerns can lead to either misclassification of a study participant for a critical window of exposure, or increased variance in the population for a given effect. Additional concerns with these types of designs for investigating health effects include the difficulty in establishing a temporal relation between exposure and effect and the introduction of recall bias when collecting information from the participants.

These study design issues are best resolved with a prospective longitudinal design, which can be accomplished for environmental chemicals during the evaluation of other factors more closely associated with pubertal development. The assessment for exposure in this setting may be more resource intensive than in

the aforementioned designs, but this can be reduced by constructing nested case-control studies. Nutrition and genetic predisposition are important contributors to growth and maturity, which require further investigations. Although it is generally accepted that nutrition affects pubertal development, unresolved issues remain because this association is variable among studies, and recent evidence suggests that birth size contributes to puberty as well (Luo et al. 2003). The extent to which birth height and body mass index relate to nutritional status in determining the timing of puberty is not known. As more work is done to resolve such questions, it would be important to control for environmental chemical exposure.

Strategies for Testing Hypotheses

Sampling Periods

The timing for the collection of exposure data (environmental, questionnaire, and biologic) depends on two general factors: the health

effect being monitored and the environmental chemical that is under evaluation. In the evaluation of sexual maturation, it is important to monitor for the formative periods of the sexual organs (*in utero*) when a significant amount of chemical exposure is expected, that is, postnatal (breast-feeding), pre- and peripuberty, and the completion of puberty. These are discussed to a greater extent elsewhere (Pryor et al. 2000). Recruitment of participants during preconception will ensure that exposures in the first trimester of gestation will be characterized, and data collected at this time may serve as a baseline comparison throughout the pregnancy and the postpartum period.

The successful completion of sexual maturation is dependent on proper anatomic development of the organs and hormonal regulation leading to their function. The anatomic development of organs and sexual differentiation of the fetus occurs during pregnancy or the *in utero* period. Organogenesis takes place in the first trimester (weeks 4–8) of gestation, and disruption during this time can lead to

Table 1. Continued

| Chemical | Reference | Developmental window or route of exposure assessed | Exposure measure | Level of exposure | Associated health effects | |
|--------------------|----------------------------|--|---|---|---|---|
| | | | | | Males | Females |
| PCBs | Blanck et al. 2000 | Intrauterine | Maternal serum level postevent ^b | Mean ± SD, 5.6 µg/L ± 5.5; range, ND ^c to 78 µg/L | | No effect on age at menarche or attainment of thelarche or pubarche by intrauterine PCB level |
| | Den Hond et al. 2002 | Puberty | Serum level at puberty | Exposures: range of PCB-138 means, 94–227 ng/L | Decreased likelihood to attain genital maturity with increasing PCB-138 level | No effect on likelihood to attain either thelarche or pubarche by PCB-138 level |
| | | | | Exposures: range of PCB-153 means, 166–332 ng/L | Decreased likelihood to attain pubarche with increasing PCB-153 level | No effect on likelihood to attain either thelarche or pubarche by PCB-153 level |
| | | | | Exposures: range of PCB-180 means, 95–202 ng/L | No effect on likelihood to attain either genital maturity or pubarche by PCB-180 level | No effect on likelihood to attain either thelarche or pubarche by PCB-180 level |
| Gladen et al. 2000 | Intrauterine | Milk level at birth | Median, 1.7 µg/g lipids, Range, 0.5–5.5 µg/g lipids | No effect on time of onset of puberty or height or body weight at puberty by PCB level from intrauterine exposure | No effect on time of onset of puberty by PCB level from intrauterine exposure Increased body weight at puberty with increasing PCB level from intrauterine exposure in Caucasian girls | |
| | | Postnatal (breastfeeding) | Lactational exposure index | | No effect on time of onset of puberty or height or body weight at puberty by PCB level from lactational exposure | No effect on time of onset, height, or body weight at puberty by PCB level from lactational exposure Premature thelarche |
| Phthalates | Colon et al. 2000 | Puberty | Serum level at puberty | Cases: DEHP, 450 µg/L (mean ± SD not given) Controls: DEHP, 70 µg/L (mean ± SD not given) | | |
| Phytoestrogens | Freni-Titulaer et al. 1986 | Puberty | Questionnaire | | | Use of soy-based formula increased likelihood for onset of premature thelarche before 2 years of age |

Abbreviations: DEHP, di-(2-ethylhexyl) phthalate; ND, not detected; *p,p'*-DDE, *p,p'*, dichlorodiphenyldichloroethylene; TEQ, toxic equivalent. ^aPBB limit of detection, 1 µg/L. ^bCows contaminated by feed containing PBB in 1973. ^cPCB limit of detection, 5 µg/L.

disorders that may be apparent at birth or not apparent until adulthood. Some notable examples of this include diethylstilbestrol (DES) and thalidomide. DES was used in the early 1940s and 1950s for the treatment of irregular vaginal bleeding and threatened abortions. DES is associated with the occurrence of cervical or vaginal clear-cell adenocarcinoma in the F₁ generation when exposure was before 18 weeks of gestation (Hatch et al. 1998). Thalidomide was used in the late 1950s to treat nausea and vomiting associated with pregnancy, causing severe limb malformations in the newborn when exposure occurred during days 34–50 of gestation (Smithells and Newman 1992). The *in utero* exposure to various stimuli leading to altered pubertal development was demonstrated in animal experiments using intrauterine artery ligation and chemical treatments. Intrauterine ligation during gestation was used to simulate malnutrition in a rat model and was shown to cause a delay in the onset of puberty in males and females (Engelbregt et al. 2000). Bisphenol A (Howdeshell et al. 1999) and octylphenol (Bogh et al. 2001; Wright et al. 2002) treatments in animals were demonstrated to advance puberty by shortening the time to either the first estrus or its equivalent. For example, the gilts of sows treated with octylphenol from days 23–85 of gestation were

observed to have a shorter period to first estrous compared with controls (mean ± SD, 211.5 ± 14.5 days vs. 246.0 ± 36.7 days) (Bogh et al. 2001). However, octylphenol did not alter testicular size in boars that were exposed to octylphenol as fetuses (Bogh et al. 2001). When ewes were treated with octylphenol from day 70 of gestation to birth, the times to the dates of first estrous and first progesterone rise in the lambs were decreased compared with controls by an average of about 20 days and 44 days, respectively (Wright et al. 2002). In the same study the lambs of ewes treated through birth and to weaning had shorter times to puberty compared with controls as well, but they were not different from lambs from ewes treated to birth. Atrazine delayed mammary gland development in female rats treated either as fetuses during gestational days 15–19 or during lactation (Rayner et al. 2004). In the same model, vaginal opening was delayed only in rat pups that were exposed to atrazine by lactation. Phytoestrogens, such as genistein (Casanova et al. 1999) and the lignan-containing flaxseed (Tou et al. 1998), advanced the time of onset of puberty in female rats when high doses were initiated during the gestational period and continued postnatally. Of note, when flaxseed was administered at a lower dose, it delayed the

onset of puberty (Tou et al. 1998). No observed pubertal effects (i.e., time of onset, testicular weight) were found in males in these phytoestrogen models. The effects of *in utero* exposure to environmental chemicals on pubertal development were evaluated in several epidemiologic studies. For PCBs and *p,p'*-DDE this route of exposure was compared with that from breast-feeding in one study (Table 1). The significant difference between these two exposure periods is that the amount of chemical delivered to the infant from lactation is usually greater than the amount of chemical delivered to the fetus by the placenta (Jacobson and Jacobson 1996). The importance of these experimental findings in animals and data on human exposure to chemicals is that they lend support to the biologic plausibility or likelihood that a chemical can either cause or modify the outcome of the purported health effect. It will be important to consider mechanisms of action, timing of exposure, and existing chemical levels in the general population from background exposure when candidate chemicals are reviewed for this hypothesis.

During the *in utero* period, the fetus should be assessed for exposures that coincide with the developmental windows of the primary and secondary sexual organs (Pryor et al. 2000) (Table 2). For example, mammary

Table 2. Sampling periods for the assessment of exposure to persistent and nonpersistent chemicals.

| | Preconception ^a | <i>In utero</i> | Perinatal | Postpartum (18–24 months) | Prepuberty (5–6 years) | Midpuberty | Postpuberty |
|--|----------------------------|-------------------------------|-----------------|---|------------------------|-----------------|-----------------|
| Persistent chemicals | | | | | | | |
| Urine ^{b,c,d} | M/F | M (10–15 weeks ^e) | M | x | x | x | x |
| Serum ^{b,f} | M/F | M (10–15 weeks ^e) | M | x | x | x | x |
| Whole blood ^{b,f} | M/F | M (10–15 weeks ^e) | M | x | x | x | x |
| Hair ^b | | | M | | | | |
| Human milk ^b | | | | 2 weeks – 2 months postpartum | | | |
| Cord serum ^b | | | x | | | | |
| Cord whole blood ^b | | | x | | | | |
| Meconium ^b | | | x | | | | |
| Dietary assessment ^g | x | M (10–15 weeks ^e) | x | x | x | x | x |
| Home air sample ^{b,g} | x | x | x | x | x | x | x |
| Home composite dust sample ^{b,g} | x | x | x | x | x | x | x |
| Other environmental samples ^{b,g} | Special studies | Special studies | Special studies | Special studies | Special studies | Special studies | Special studies |
| Questionnaire ^g | x | x | x | — At 6, 12, 24 and 36 months, then annually until puberty — | | | |
| Ecologic analysis (e.g., GIS) ^g | x | x | x | x | x | x | x |
| Nonpersistent chemicals | | | | | | | |
| Urine ^{b,c,d} | M/F | M (10–15 weeks ^e) | M | x | x | x | x |
| Serum ^{b,f} | M/F | M (10–15 weeks ^e) | M | x | x | x | x |
| Human milk ^b | | | | 2 weeks – 2 months postpartum | | | |
| Cord serum ^b | | | | x | | | |
| Meconium ^b | | | | x | | | |
| Dietary assessment ^g | x | M (10–15 weeks ^e) | x | x | x | x | x |
| Home air sample ^{b,g} | x | x | x | x | x | x | x |
| Home composite dust sample ^{b,g} | x | x | x | x | x | x | x |
| Other environmental samples ^{b,g} | Special studies | Special studies | Special studies | Special studies | Special studies | Special studies | Special studies |
| Questionnaire ^g | x | x | x | — At 6, 12, 24 and 36 months, then annually until puberty — | | | |
| Ecologic analysis (e.g., GIS) ^g | x | x | x | x | x | x | x |

GIS, geographic information system; F, father; M, mother; x, period when samples are to be collected.

^aAt the time of enrollment. ^bMedia with extant laboratory methods for likely target chemical agent. Note that for nonpersistent chemicals, multiple samples of biologic specimens may be needed during this time because of possible variability in biologic exposure level due to the short half-life of the chemical. This is particularly important if a critical exposure period is being considered. Alternatively, pilot data may demonstrate the lack of need for frequent sampling if constant exposure occurred in a stable environment. ^cTimed specimen collection; morning void specimen with creatinine measurement. ^dPediatric urine bag or diaper sample for non-toilet-trained children. If not diaper, spot samples or multiple spots. ^eFor example, weeks 10–15 of gestation are a critical period for breast development. ^fWhen blood collection is at a young age, piggyback on lead screen at 12 and 24 months that is recommended by the Centers for Disease Control and Prevention. ^gEnvironmental sampling, dietary assessment, questionnaires, and ecologic analysis are necessary for persistent chemicals to determine either route or pathway of exposure; otherwise, exposure can be established through the analysis of biologic specimens. Information should be obtained proximate to the sampling period for biologic specimens.

gland development begins at about week 4 and continues through week 10 of gestation. The primary mammary bud is formed during this period. From weeks 10 through 15 of gestation, formation of secondary buds, the nipple, and the areola occurs. The critical window of development for the external genitalia is from weeks 7 through 12 of gestation. Because it is important to assess for exposure during this early period of gestation, it is recommended to recruit study participants before conception to ensure that this critical period during gestation is not missed.

Once the sexual organs are formed, their activation for reproduction is determined by hormonal (gonadotropins, estrogens, androgens) regulation. In females this is largely determined by estrogenic activity and in males, by androgens. In the prepubertal female, estrogen levels are low and stable until puberty, when a surge in estrogen level occurs, resulting from gonadotropin stimulation. The pituitary gland begins to secrete luteinizing hormone at approximately 1–3 years before the onset of puberty. The prepubertal period is an important time to assess for environmental chemical exposure because of several reported cases of girls developing premature thelarche after being exposed to estrogen compounds (Teilmann et al. 2002). Thus, the sampling would include *in utero* (gestation), postnatal (for breast-feeding infants), before puberty (pre- and peripuberty), and postpuberty (or the completion) periods. It is important to monitor puberty to its completion because the time of onset of puberty and the duration for its completion may vary independently. Further definitions of the frequency for monitoring during the prepubescent period may be gained from reviewing longitudinal studies on puberty (Biro et al. 1995; Lee 1980; Roche et al. 1995).

Populations with High Exposures

Certain populations that may be susceptible or vulnerable to environmental chemical exposure will need to be considered in the study design. The reasons are variable and include genetic predisposition, nutrition, and socioeconomic factors. For example, persons frequently ingesting fish or whale meals are likely to have elevated levels of PCBs, polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs). This was observed in persons residing near the Great Lakes region of the United States and members of the Inuit tribe in Canada. Children residing in homes built before 1946 were found to have approximately a 5-fold increased incidence of blood lead levels $\geq 10 \mu\text{g/dL}$ compared with children residing in homes built after 1973 (Pirkle et al. 1998). Low-income status and African-American race/ethnicity were associated with elevated blood lead levels ($\geq 10 \mu\text{g/dL}$) in families living in homes built before 1946.

Environmental Chemicals

Many environmental chemicals are observed to have endocrine or hormonal activity (National Research Council 1999). However, only a few of these chemicals are associated with effects on the age at puberty in humans, including lead (Selevan et al. 2003; Wu et al. 2003), PBBs (Blanck et al. 2000), PCBs (Den Hond et al. 2002; Gladen et al. 2000), PCDDs/PCDFs (Den Hond et al. 2002), *p,p'*-DDT/*p,p'*-DDE (Gladen et al. 2000; Krstevska-Konstantinova et al. 2001), endosulfan (Saiyed et al. 2003), phthalates (Colon et al. 2000), and phytoestrogens (Freni-Titulaer et al. 1986). To encompass all these chemicals into the assessment strategy, the chemicals can be categorized as either biologically persistent or nonpersistent (Table 2). Generally, persistent chemicals have long half-lives (months to years) and are measured in the blood. Although this may seem advantageous from a sampling perspective (i.e., wide window of opportunity), it does pose a challenge when critical windows of exposure are of concern and when subacute exposures may have occurred. Thus, if exposure occurs during critical periods of a child's development, then specimens should be obtained proximate to those periods. Questionnaires and environmental assessment would be necessary if the pathways of exposure are important, because biologic monitoring would not be able to identify the exposure pathway or the exposure location. For example, the presence of a persistent chemical in the blood of a person who recently moved to a new location would most likely represent an exposure from the former location. However, this would not be known by the blood test alone. If documentation of exposure of a persistent chemical is all that is necessary, then biologic monitoring should be adequate. Nonpersistent chemicals have short half-lives (hours to days) and are usually measured in the urine. For nonpersistent organic chemicals, increased reliance is needed on questionnaires and environmental samples to document exposure. This is because of the relatively short time that these chemicals exist in the body.

Measurements

Exposure assessment. The choice of the biologic matrix to measure for the chemicals is largely determined by the properties of the chemical (e.g., lipid solubility, protein-binding capacity, and ionic charge), which governs the distribution of the chemical in the body. Blood and urine are the standard specimens used for the measurement of chemicals, and water-soluble chemicals are commonly quantified in urine. In addition, the target organ or organism needs to be considered. For example, if the interest is in fetal exposure, then cord blood is the preferred specimen. If the route of exposure is

important, for example, identify either the source of exposure or the degree to which one source may be more important in determining a health outcome. If two sources are involved, then several matrices will have to be sampled. For example, an infant's exposure can occur either during the *in utero* period through the placenta or during breast-feeding. However, neither the amount of chemical exposure nor the determined health effect is consistent by either of these routes of exposure. In the case of PCB exposure, it was determined that breast-feeding contributed to a larger amount of exposure to the infant than did placental transfer; however, the latter was deemed more consequential to delayed neurodevelopment (Jacobson and Jacobson 1996). If the extent to which a chemical partitions into various biologic matrices is known, then alternative specimens may be used if the desired specimen is not adequate. For example, maternal blood, cord blood, and maternal milk may be used to assess for fetal exposure to persistent organic chemicals. The assessment of the infant to exposure to chemicals from human milk requires multiple milk samplings during the breast-feeding period because of the expected decline in the level of persistent chemicals in human milk from maternal depuration. Additional consideration is needed to characterize the difference between infants who are exclusively breast-fed and those who are partially breast-fed because the volume of milk intake will vary by these practices of breast-feeding. Meconium is a biologic matrix that is currently under investigation as an indicator of chemical exposure. The interest in this matrix is because of its potential to reflect chemical exposures to the fetus as early as the second trimester.

Researchers may need to customize questionnaires and environmental sampling based on the use patterns of particular classes of chemicals. Phthalates, for example, are

Table 3. Health effect measures to assess for pubertal development.^a

| |
|--|
| Onset of puberty |
| Sexual maturity rating (Tanner staging) |
| Performed by observer or by self-reporting |
| Frequency: annually |
| Stature |
| Hormones [luteinizing hormone, estradiol, testosterone, dehydroepiandrosterone-sulfate (DHEA-S)] |
| Salivary hormone levels |
| Thyroid-stimulating hormone |
| Later stages of puberty |
| Voice pattern change (males) |
| Menarche |
| Menstrual history |
| Dual-energy X-ray absorptiometry |
| At age 6 and 12 years for females and at age 6 and 14 years for males |

^aDiscussed at the NCS inter-work group meeting "Obesity and Physical Development." This meeting was co-chaired by the Nutrition, Growth, and Pubertal Development and the Exposure to Chemical Agents working groups in Baltimore, Maryland (USA), on 17–18 December 2002.

commonly found in personal care products (e.g., cosmetics, shampoos); therefore, researchers should ask specific questions regarding the use of these products (Tiwary and Ward 2003). Similarly, changes in location throughout the life stages (home, school, work site) may warrant adjustments of these assessment tools. In the design of these exposure assessment tools, it is important to validate them before applying them to health effect studies.

Biologic markers of health effect. These measurements will be used to monitor the onset and completion of puberty. Various tools can be used, including biochemical measures (e.g., hormones), physical examination, and diagnostic scans (Rockett et al. 2004) (Table 3). Dual-energy X-ray absorptiometry is a noninvasive method to assess body fat mass, body fat-free mass, and bone mineral mass and density for skeletal maturity.

Potential Risk Factors for Altered Pubertal Development

Several factors are known to cause or are associated with altered pubertal development that need to be considered in the study because they may bias the estimate of the relationship between chemical exposure and the age of onset of puberty. Some of these items are listed in Table 4.

Conclusion

Evidence exists that nutritional status, genetic predisposition (race/ethnicity), and environmental chemical exposure are associated with altered age at puberty. The assessment for exposure to environmental chemicals that may

affect the attainment of puberty in the developing child is challenged by several factors, including the various developmental periods when an exposure can affect the maturation process, limited access to certain biologic specimens during select periods of human development, and the many chemicals with varying properties. The recommended approach is to conduct the assessment by life stages (i.e., *in utero*, postnatal, pre- and peripubertal). Study participants need to be recruited at preconception to ensure that the “critical window” of gestation, that is, the first trimester, is included in the exposure assessment process. Chemicals should be categorized by biologic persistence, and biologic specimens that are most likely to yield meaningful information should be collected and stored. The latter will allow for the judicious use of specimens of limited quantity for special investigations (e.g., nested case-control studies) and time to develop new and improved analytical methods. For chemicals that either cannot be measured in biologic specimens or have short biologic half-lives, the analysis of environmental samples and use of questionnaire data are necessary to complete the assessment for exposure. Food and dietary data may assist in determining the extent to which nutrients and chemicals from this pathway contribute to the variance in the timing of puberty. Factors that are either known causes of, or associated with, altered pubertal development need to be controlled for during the assessment of health outcomes. The National Children’s Study is uniquely poised to evaluate the effects of environmental chemicals on the age at puberty, and the above approach will allow the NCS to accomplish this task.

Table 4. Potential risk factors affecting pubertal development.

| |
|--|
| Precocious |
| Gonadotropin-dependent (central precocious puberty) |
| Brain pathology (e.g., hypothalamic hamartoma, tumors, hydrocephalus, severe head trauma) |
| Hypothyroidism, untreated |
| Gonadotropin-independent precocious puberty |
| McCune-Albright syndrome in girls |
| Familial male precocious puberty (testotoxicosis) |
| Tumors (ovarian, adrenocortical, Leydig cell, chorionic gonadotropin-secreting tumors) |
| Exogenous pharmaceutical estrogen or androgen use |
| Isolated premature thelarche, premature pubarche/adrenarche, premature menarche |
| Delayed |
| Poor nutrition |
| Chronic illness |
| Constitutional growth delay |
| Intense physical training |
| General categories associated with altered pubertal development |
| General nutrition |
| General health |
| Brain injury |
| Obesity |
| Socioeconomic status |
| Immigration/adoption |
| Race/ethnicity |
| Pharmaceuticals [estrogenic, androgenic, estrogen blocker (i.e., aromatase inhibitors), and androgen blocker (e.g., finasteride, flutamide)] |
| Genetics |
| Gestational age |

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